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=> index bioscience chemistry
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       BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT,
       CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE,
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91 FILES IN THE FILE LIST IN STNINDEX

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=> s (troponin same (preparing or preparation or isolation or isolating or purification
or purifying)) and (sulfhydryl same (protecting or protected )) and (sodium (w)sulfite)
MISSING OPERATOR 'SAME (PREPARING'
The search profile that was entered contains terms or
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=> s (troponin (s) (preparing or preparation or isolation or isolating or purification or
purifying)) and (sulfhydryl same (protecting or protected )) and (sodium (w) sulfite)
MISSING OPERATOR 'SAME (PROTECTING'
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s (troponin (s) (preparing or preparation or isolation or isolating or purification or
purifying)) and (sulfhydryl (s)  (protecting or protected )) and (sod
UNMATCHED LEFT PARENTHESIS 'AND (SOD'
The number of right parentheses in a query must be equal to the
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=> s (troponin (s) (preparing or preparation or isolation or isolating or purification or
purifying)) and (sulfhydryl (s)  (protecting or protected )) and (sodium (w) sulfite)
          1   FILE BIOTECHABS
          1   FILE BIOTECHDS
  13 FILES SEARCHED...
          1   FILE CAPLUS
  24 FILES SEARCHED...
          0*  FILE FEDRIP
  39 FILES SEARCHED...
          2   FILE IFIPAT
  51 FILES SEARCHED...
          2   FILE USPATFULL
  63 FILES SEARCHED...
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  90 FILES SEARCHED...

   5 FILES HAVE ONE OR MORE ANSWERS,   91 FILES SEARCHED IN STNINDEX

L1   QUE (TROPONIN (S) (PREPARING OR PREPARATION OR ISOLATION OR ISOLATING OR P
         URIFICATION OR PURIFYING)) AND (SULFHYDRYL (S) (PROTECTING OR PROTECTE
         D )) AND (SODIUM (W) SULFITE)

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                                                           ENTRY    SESSION
FULL ESTIMATED COST                                        33.92      34.13

FILE 'IFIPAT' ENTERED AT 13:42:54 ON 19 DEC 2002
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FILE 'USPATFULL' ENTERED AT 13:42:54 ON 19 DEC 2002
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FILE 'BIOTECHDS' ENTERED AT 13:42:54 ON 19 DEC 2002
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L2 2 FILE IFIPAT
L3 2 FILE USPATFULL
L4 1 FILE BIOTECHDS
L5 1 FILE CAPLUS

TOTAL FOR ALL FILES
L6 6 L1

=> d l6 1-6 ibib abs

L6 ANSWER 1 OF 6 IFIPAT COPYRIGHT 2002 IFI
AN 10121228 IFIPAT;IFIUDB;IFICDB
TITLE: **PURIFICATION** OF HUMAN **TROPONIN** I
INVENTOR(S): Conn; Gregory, Cary, NC, US
Reardon; Brian, Seattle, WA, US
Zeng; Xianfang, Northborough, MA, US
Zhang; Chenming, Blacksburg, VA, US
PATENT ASSIGNEE(S): Diosynth RTP, Inc.
AGENT: DARBY & DARBY P.C., 805 Third Avenue, New York, NY, 10022, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2002064835	A1	20020530
APPLICATION INFORMATION:	US 2001-903398		20010710

	NUMBER	DATE
	US 2000-217069	20000710 (Provisional)
FAMILY INFORMATION:	US 2002064835	20020530

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION
NUMBER OF CLAIMS: 20 11 Figure(s).

DESCRIPTION OF FIGURES:
FIGS. 1A and 1B. A. Proposed reaction for oxidative sulfitolysis. B. Cleavage of disulfide bond by **sodium sulfite** to form the Ssulfo derivative.
FIG. 2. **Preparation** and washing of TnI-containing inclusion bodies.
FIG. 3. Summary of rTroponin-I **preparation**.
FIG. 4. Q-Sepharose FF chromatography of **Troponin** I. Buffer A: 6 M urea, 25 mM Tris-HCl, pH 7.5, 100 mM; Buffer B: 6 M urea, 25 mM Tris-HCl, pH 7.5, 2 M NaCl; Gradient: Step, 0% B for the flow-through and 100% B for the strip; and Flow rate: 150 ml/ min.
FIG. 5. 300 ml Q-sepharose FF chromatography. Buffer A: 6 M urea, 25 mM Tris-HCl, pH 7.5, 100 nM; Buffer B: 6 M urea, 25 mM TrisHCl, pH 7.5, 2 M NaCl; Gradient: Step, 4% B for elution and 50% B for strip; and Flow rate: 20 ml/min.
FIG. 6. SDS-PAGE analysis **troponin** lot after anion exchange steps no.

1 and no. 2 in 16% tris-glycine gel, under nonreducing conditions. A-H refer to lanes in the SDS-PAGE gel. A. Sulfitolyzed **troponin** Lot 3L4 standard; B. solubilized inclusion bodies; C. sulfitolyzed inclusion bodies (AEX No. 1 load); D. anion exchange no. 1 flowthrough; E. anion exchange no. 1 salt eulate; F. anion exchange no. 2 load; G. anion exchange no. 2 flowthrough; and, H. anion exchange no. 2 100 mM NaCl eluate.

FIG. 7. Toyopearl 650 M (phenyl) HIC chromatograph. Buffer A: 6 M urea, 25 mM Tris-HCl, pH 7.5, 1 M (NH4)2SO4; Buffer B: 6 M urea, 25 mM Tris-HCl, pH 7.5; Gradient: Step, 100% B for the flow-through and 0% B for strip; and Flow rate: 10 ml/min.

FIGS. 8. SDS-PAGE analysis **troponin** lot after hydrophobic interaction chromatography in 16% tris-glycine gel, under nonreducing conditions. A-F refers to lanes in the SDS-PAGE gel. A. Sulfitolyzed **troponin** Lot 3L4 standard; B. AEX step no. 2, **troponin** eulate pool; C. HIC load (w/1M ammonium sulfate); D. HIC flowthrough (**troponin** product); E. HIC low salt eulate (column strip); F. lot 3L5 sulfitoylzed **troponin** product.

FIG. 9. Quantitation of rTnI on Zorbax C3.

FIG. 10. **Troponin** I LysC mapping.

FIG. 11. SD S-PAGE analysis of sulfitolyzed **troponin** reduction with dithiothreitol for 45 mins. at ambient temperature. One mg/ ml TnI in 6 M urea, 25 mM tris, 0.15 M NaCl pH 7.5, run on 16% tris-glycine gel. 1. 10., Mark 12 MW Stds; 2. 9., sulfitolyzed TnI; 3. 0.05 mM DTT; 4. 0.10 mM DTT; 5. 0.2 mM DTT; 6. 0.3 mM DTT; 7. 0.5 mMDTT; 8. 1.0 mM DTT.

AB The invention is directed to methods for **purifying** **Troponin** I, particularly recombinant Tropnin I produced in a bacterial expression system. Recombinant Tropnin I can be advantageously purified after reversibly **protecting** the free **sulfhydryl** groups, e.g., by forming sulfates. In a specific example, Tropnin I reacted with sodium tetrafhionate yielded sulfitolyzed Tropnin I, which was purified by chromatography on an anion exchanger, followed by hydrophobic interaction chromatography. Facile deprotection of the **sulfhydryl** groups yields a highly purified product ready for refolding.

CLMN 20 11 Figure(s).

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L6 ANSWER 2 OF 6 IFIPAT COPYRIGHT 2002 IFI

AN 10111538 IFIPAT;IFIUDB;IFICDB
TITLE: **PURIFICATION** OF HUMAN **TROPONIN** I
INVENTOR(S): Conn; Gregory, Cary, NC, US
Reardon; Brian, Seattle, WA, US
Zeng; Xianfang, Northborough, MA, US
Zhang; Chenming, Blacksburg, VA, US
PATENT ASSIGNEE(S): Diosynth RTP, Inc.
AGENT: DARBY & DARBY P.C., 805 Third Avenue, New York, NY, 10022, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2002055145	A1	20020509
APPLICATION INFORMATION:	US 2001-998619		20011130

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
CONTINUATION OF:	US 2001-903398	20010710	PENDING

	NUMBER	DATE
	US 2000-21706920000710	(Provisional)
FAMILY INFORMATION:	US 2002055145	20020509

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION
NUMBER OF CLAIMS: 20 11 Figure(s).

DESCRIPTION OF FIGURES:
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CLMN 20 11 Figure(s).

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mM DTT; 5. 0.2 mM DTT; 6. 0.3 mM DTT; 7. 0.5 mMDTT; 8. 1.0 mM DTT.

L6 ANSWER 3 OF 6 USPATFULL

ACCESSION NUMBER:	2002:126323 USPATFULL
TITLE:	**Purification** of human **troponin** I
INVENTOR(S):	Conn, Gregory, Cary, NC, UNITED STATES Reardon, Brian, Seattle, WA, UNITED STATES Zeng, Xianfang, Northborough, MA, UNITED STATES Zhang, Chenming, Blacksburg, VA, UNITED STATES
PATENT ASSIGNEE(S):	Diosynth RTP, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002064835	A1	20020530
APPLICATION INFO.:	US 2001-903398	A1	20010710 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-217069P	20000710 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	DARBY & DARBY P.C., 805 Third Avenue, New York, NY, 10022
NUMBER OF CLAIMS:	20
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	11 Drawing Page(s)
LINE COUNT:	566

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention is directed to methods for **purifying** **Troponin** I, particularly recombinant Tropnin I produced in a bacterial expression system. Recombinant Tropnin I can be advantageously purified after reversibly **protecting** the free **sulfhydryl** groups, e.g., by forming sulfates. In a specific example, Tropnin I reacted with sodium tetrafhionate yielded sulfitolyzed Tropnin I, which was purified by chromatography on an anion exchanger, followed by hydrophobic interaction chromatography. Facile deprotection of the **sulfhydryl** groups yields a highly purified product ready for refolding.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 4 OF 6 USPATFULL

ACCESSION NUMBER:	2002:105940 USPATFULL
TITLE:	**Purification** of human **troponin** I
INVENTOR(S):	Conn, Gregory, Cary, NC, UNITED STATES Reardon, Brian, Seattle, WA, UNITED STATES Zeng, Xianfang, Northborough, MA, UNITED STATES Zhang, Chenming, Blacksburg, VA, UNITED STATES
PATENT ASSIGNEE(S):	Diosynth RTP, Inc. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002055145	A1	20020509
APPLICATION INFO.:	US 2001-998619	A1	20011130 (9)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 2001-903398, filed on 10 Jul 2001, PENDING		

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-217069P	20000710 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	DARBY & DARBY P.C., 805 Third Avenue, New York, NY, 10022
NUMBER OF CLAIMS:	20

EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 11 Drawing Page(s)
LINE COUNT: 570
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention is directed to methods for **purifying** **Troponin** I, particularly recombinant Tropnin I produced in a bacterial expression system. Recombinant Tropnin I can be advantageously purified after reversibly **protecting** the free **sulfhydryl** groups, e.g., by forming sulfates. In a specific example, Tropnin I reacted with sodium tetrafhionate yielded sulfitolyzed Tropnin I, which was purified by chromatography on an anion exchanger, followed by hydrophobic interaction chromatography. Facile deprotection of the **sulfhydryl** groups yields a highly purified product ready for refolding.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 5 OF 6 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-08599 BIOTECHDS
TITLE: **Purifying troponin** I comprises subjecting **troponin** I to chromatography on anion exchanger after reversibly **protecting** the free **sulfhydryl** groups;
recombinant production in Escherichia coli and application in e.g. cancer therapy
AUTHOR: CONN G; REARDON B; ZENG X; ZHANG C
PATENT ASSIGNEE: DIOSYNTH RTP INC
PATENT INFO: WO 2002004512 17 Jan 2002
APPLICATION INFO: WO 2000-US21817 10 Jul 2000
PRIORITY INFO: US 2000-217069 10 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-154921 [20]
AN 2002-08599 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - **Preparing troponin** I, comprising **protecting** free **sulfhydryl** groups of **troponin** I under reducing conditions, and **troponin** I is then purified by subjecting **troponin** I comprising **sulfhydryl** **protecting** groups to chromatography, is new.
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for **troponin** I comprising **sulfhydryl protecting** groups.
BIOTECHNOLOGY - Preferred Method: The recombinant **troponin** I is expressed in a bacterial expression system, preferably an Escherichia coli expression system. The free **sulfhydryl** groups are **protected** by sulfitolyzation which comprises reacting reduced recombinant **troponin** I with sodium tetrathionate. **Troponin** I is purified by chromatography under non-reducing conditions and the **sulfhydryl** groups are deprotected from the purified **troponin** I. The chromatographic support is an anion exchange column, optionally followed by hydrophobic interaction chromatography. **Troponin** I is denatured and the **sulfhydryl protecting** groups are sulfates.
ACTIVITY - Cytostatic.
MECHANISM OF ACTION - Inhibitor of angiogenesis. No supporting data is given.
USE - The method is useful for **purifying troponin** I, particularly recombinant **troponin** I. The highly purified **troponin** I, preferably in a refolded state is useful for antibody generation, as a control or standard immunoassay reagent, or to inhibit angiogenesis important in treating various cancers.
ADVANTAGE - Protection of **sulfhydryl** groups during **troponin** I **preparation** eliminates the costly need for maintaining non-reducing conditions throughout protein

preparation, **purification** and storage, and need for reducing agents. The **sulfhydryl-protected** **troponin** does not form intrachain or interchain disulfide crosslinks. Overall yield of **troponin** from the multi-step **purification** was greater than 50% at purity levels of greater than 95%. Deprotection of the **sulfhydryl** groups yields a highly purified product ready for refolding.

EXAMPLE - Human skeletal **troponin** I (TnI) expressed in Escherichia coli was isolated from lysed cells in inclusion bodies. 10 g of TnI-containing inclusion bodies were solubilized and protein **sulfhydryls** were sulfitolyzed using 6 M urea (200 ml), Tris (25 mM), **sodium sulfite** (10 mg/ml), sodium tetrathionate (5 mg/ml) pH 7.5 at ambient temperature for 6 hours in the dark. The solubilized material was filtered over a 0.2 micro membrane prior to subsequent processing. Sulfitolyzed recombinant human TnI was purified by a five step process. Solubilized, sulfitolyzed TnI-containing inclusion bodies (200 ml) were loaded onto a 3 l volume Q-sepharose FF column pre-equilibrated in 6 M urea, 25 mM Tris, 0.1 M NaCl pH 7.5 at 150 ml/min. The purified TnI was collected in the column flowthrough. The recovered TnI was concentrated. This material was loaded onto a 300 ml volume Q-sepharose FF column pre-equilibrated in 6M urea, 25 mM Tris, pH 7.5 at 20 ml/minute. The bound TnI was eluted from the column by a step wash with 6 M urea, 25 mM Tris, 80 mM NaCl pH 7.5. This eluted **troponin** (500 ml) was loaded onto a 60 ml column of Toyopearl 650 M phenyl HIC resin after addition of ammonium sulfate to a final concentration of 1 M. The column was pre-equilibrated with 6 M urea, 25 mM Tris, 1M ammonium sulfate pH 7.5. The purified **troponin** was collected as the unbound flowthrough from this column, concentrated 2.5-fold and buffer exchanged for storage by UF/DF. Purified TnI was stored frozen at -70 degrees C. Protein purity was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and reverse phase chromatography and protein identity was confirmed by peptide mapping with peptide mass and fragmentation analysis. Yield determinations for each step were determined by quantitative reverse phase chromatography. Residual DNA levels, measured by DNA threshold, were less than or equal to 12 pg DNA/mg protein. Endotoxin testing of final product by Limulus Amoebocyte Lysate (LAL) (gel-clot) indicated less than or equal to 3 EU/mg protein. (28 pages)

L6 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:51523 CAPLUS
DOCUMENT NUMBER: 136:101258
TITLE: Chromatographic **purification** of human **sulfhydryl-protected** recombinant **troponin** I
INVENTOR(S): Conn, Gregory; Reardon, Brian; Zeng, Xiangang; Zhang, Chenming
PATENT ASSIGNEE(S): Diosynth RTP, Inc., USA
SOURCE: PCT Int. Appl., 28 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002004512	A2	20020117	WO 2001-US21817	20010710
WO 2002004512	A3	20020516		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, EC, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 2001073348	A5	20020121	AU 2001-73348	20010710
US 2002064835	A1	20020530	US 2001-903398	20010710
US 2002055145	A1	20020509	US 2001-998619	20011130

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	US 2001-903398	A1	20010710
	WO 2001-US21817	W	20010710

AB The invention is directed to methods for **purifying** **troponin** I, particularly recombinant **troponin** I produced in a bacterial expression system. Recombinant troponin I can be advantageously purified after reversibly **protecting** the free **sulfhydryl** groups, e.g. by forming sulfates. In a specific example, troponin I reacted with sodium tetrathionate yielded sulfitolyzed troponin I, which was purified by chromatog. on an anion exchanger, followed by hydrophobic interaction chromatog. Facile deprotection of the sulfhydryl groups yields a highly purified product ready for refolding.

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